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TITLE: Tumor-Targeted Silencing of Bcl-2/Bcl-Xl by Self-Assembled Sirna-Nanovectors as a Novel Molecular Therapy for Breast Cancer

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14. ABSTRACT
The major goal of this Concept Award project is to explore the anti-Her-2 antibody as targeting ligand to establish the self-assembled nanovectors for targeted siRNA delivery to Her-2(+) breast cancer. Our hypothesis is (1) anti-Her-2 antibody will specifically target nanovectors to Her-2(+) breast cancer and thus efficiently deliver siRNA to the target cells; (2) siRNA-nanovectors will potently silence the Bcl-2/Bcl-xL gene expression, induce apoptosis in human breast cancer cells that depend on Bcl-2/Bcl-xL for survival. Since the start of this project, we have tested various conditions for the self-assembled nanovectors, based on our patented nanotechnology platform. However, we have met with technical difficulty using original technique of non-covalent complexing, some modification is needed to make Herceptin-nanovectors. Therefore, we requested and obtained approval a one-year no cost extension to optimize the conditions for nanovectors. Based on the data obtained in this first year, the anti-Her-2-nanovector shows increased transfection efficiency in Her-2(+) breast cancer cells, with 2-5-fold increase of reporter gene expression as compared with non-targeted one. We have also designed and validated siRNA/shRNA on Bcl-2 and Bcl-xL that can knockdown target genes expression up to 90%. These data provide us the foundation to accomplish the project in the second year.

15. SUBJECT TERMS
Her-2/neu, Bcl-xL, Bcl-2, Herceptin, siRNA

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I. Introduction:

The major goal of this Concept Award project is to explore the anti-Her-2 antibody as a targeting ligand to establish the self-assembled nanovectors for targeted siRNA delivery to Her-2(+) breast cancer. Our *hypothesis* is (1) anti-Her-2 antibody will specifically target nanovectors to Her-2(+) breast cancer and thus efficiently deliver siRNA to the target cells; (2) siRNA-nanovectors will potently silence the Bcl-2/Bcl-xL gene expression, induce apoptosis in human breast cancer cells that depend on Bcl-2/Bcl-xL for survival. We will prepare and optimize the anti-Her-2-nanovectors for efficient Bcl-2/Bcl-xL siRNA delivery to human breast tumors both *in vitro* and *in vivo* in nude mouse xenograft models. Successfully carried out, our studies will provide the proof-of-concept that siRNA can be delivered by the self-assembled nanovectors for tumor-targeted silencing of the genes critical for breast cancer progression and survival.

II. Research progress and key research acomplishments:

This project is one-year Concept Award project. Since the start of this project, we have tested various conditions for the self-assembled nanovectors, based on our patented nanotechnology platform. However, we have met with technical difficulty using original technique of non-covalent complexing, some modification is needed to make Herceptin-nanovectors. Therefore, we requested and obtained approval a one-year no cost extension to optimize the conditions for nanovectors. Based on the data obtained in this first year, the anti-Her-2-nanovector shows increased transfection efficiency in Her-2(+) breast cancer cells, with 2-5-fold increase of reporter gene expression as compared with non-targeted one. We have also designed and validated siRNA/shRNA on Bcl-2 and Bcl-xL that can knockdown target genes expression up to 90%. These data provide us the foundation to accomplish the project in the second year.

Specifically, we carried out the following studies:

Task 1. Preparation of the anti-Her-2-nanovectors for efficient siRNA delivery to human breast cancer cells both *in vitro* and *in vivo* in nude mouse xenograft models.

1.1. Prepare the anti-Her-2-nanovectors encapsulating siRNA using the anti-Her-2 antibody Herceptin.

1.2. Optimize the composition of siRNA-nanovectors for efficient siRNA delivery in various Her-2(+) breast cancer cell lines, including MCF-7-Her-2 stable line.

We have tested various conditions for the self-assembled nanovectors, based on our patented nanotechnology platform. However, we have met with technical difficulty using original technique of non-covalent complexing, some modification is needed to make Herceptin-nanovectors.

We have prepared a series of Herceptin-nanovectors with various of compositions, and are testing the transfection efficiencies in the breast cancer cell lines with Her-2/neu overexpression, i.e., BT-474, SK-BR-3, as well as MCF-7-Her-2 (MCF-7-H18) which was transfected with human Her-2/neu gene, versus the Her-2 negative MDA-231 cells.

Figure 1 shows one set of representative results of Herceptin-nanovector transfection experiments, based on our scFv-nanovector technology published previously (1-4). The ratio of LipA:Ab (w/w) at 3:1 seems best in both cell lines to terms of transfection efficiency for the Her-2(+) breast cancer cells. It gave 2-5-fold increase of the reporter expression comparing with LipA only without antibody. The data are consistent in two independent experiments. We are now testing the nanovectors in other cell lines. The data lay the foundation to move into in vivo studies.

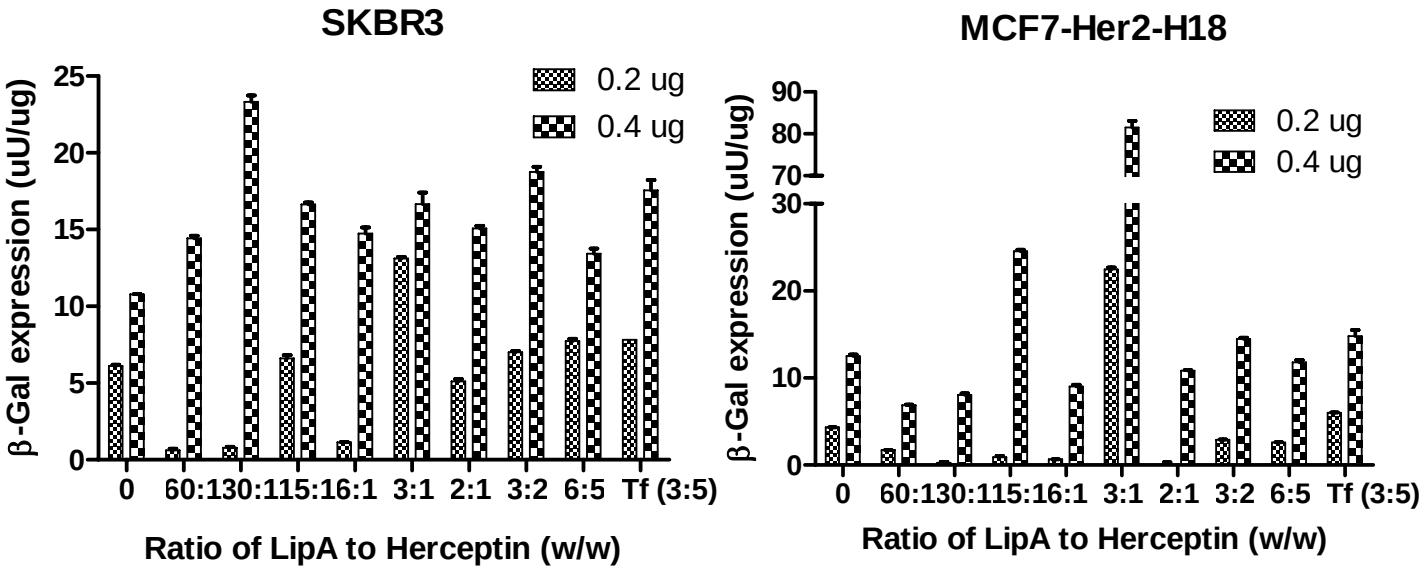

Figure 1. Anti-Her-2 antibody increases the transfection efficiencies of nanovectors (immunolipoplexes) in Her-2(+) breast cancer cells SK-BR-3 (**A**) and MCF-7-Her2-H18 (**B**). Transfection efficiency was assessed using β-*gal* as the reporter gene. Plasmid DNA containing β-*gal* gene under CMV promoter was diluted in serum-free media and added to the LipA-Herceptin complex at a DNA:lipid ratio of 1:10 (ug/nmol). The solution was mixed well for 10 min by inversion several times to produce the LipA-Herceptin-DNA complex. The *in vitro* transfection was performed in 24-well plates. Forty-eight hours after transfection, the cells were washed with PBS and lysed to measure the β-Gal gene expression and protein concentration. The β-*gal* enzymatic assay (Promega, E2000) was used, and results were shown as microunits of β-*gal*/ug protein. The data was representative of two independent experiments.

1.3 Design and validation of siRNA on Bcl-2 and Bcl-xL

We have designed a series of siRNA and shRNA for Bcl-2 and Bcl-xL and validated them in cancer cells. All siRNAs show very potent gene knock-down on Bcl-2 or Bcl-xL, up to >90% knock-down (data not shown).

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